also the point at which the ends of the thick filaments should collide with the Z lines, thereby possibly producing a resistance to shortening, and if the thick filaments crumple or fold, the number of cross-bridges capable of generating force might be reduced (*2*). The disadvantage of using this to explain our observation is that one must postulate differences in length or compressibility of the filaments at different points along a radius of the fiber, for which, as far as we know, there is no evidence. Differences along a radius would also be required for an explanation related to an increase in the filament lattice spacing with shortening below slack length (*13*), or to an increase in the internal osmotic pressure (*2*), to the exhaustion of some energy supply, or to a facilitated removal of calcium.

Another possibility is that shortening somehow interrupts the inward spread of activation along the transverse tubular (T) system (*14*), or that some property of the T-system differs in different parts of a fiber. For example, the fact that the lumina of the tubules are two to three times larger near the fiber surface than they are deeper in the fiber may be significant (*15*). This last possibility is supported by the observation that the electrical event producing activation apparently spreads along the T-system near the fiber surface more readily than toward the center of the fiber under certain circumstances (*16*). We have also observed the pattern of shortening produced by immersing fibers in 1.5 m*M* caffeine, which is believed to penetrate the fiber membrane and to produce a contraction by releasing calcium from the sarcoplasmic reticulum (*17*). We found that a fiber in caffeine contracture shortens to well below 1.6 μm with no wavy fibrils in evidence (Fig. 3). This agrees with the possibility that the waviness induced by shortening to 1.6 μm in response to membrane depolarization is related to an interruption of the inward spread of the activating signal or perhaps to uncoupling between the T-system and the sarcoplasmic reticulum.

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Harderian Gland: An Extraretinal Photoreceptor Influencing the Pineal Gland in Neonatal Rats?

Abstract. *The circadian rhythm of pineal serotonin and the influence of light on that rhythm have been confirmed. Removal of the Harderian gland abolishes the response to light in blinded animals, which suggests that this gland may act as the extraretinal transducer involved in the persistence of the pineal rhythm in blinded suckling rats.*

The serotonin content of the pineal gland undergoes a circadian rhythm with a maximum at about 1 p.m. and a minimum at 11 p.m. when lights are on from 5 a.m. to 7 p.m. (*1*). This rhythm persists in attenuated form in blinded animals and in rats kept in continuous darkness for up to 2 weeks (*2, 3*), but the nocturnal decline can be prevented if light is extended an additional 4 hours to 11 p.m. (*1, 3*). This effect of light on pineal serotonin occurs in blinded animals at 12 days of age (*4*), but not in blinded adults, which suggests the existence of a nonretinal photoreceptor influencing the pineal in suckling rats. Further, this receptor was localized in the head by the observation that hooding completely abolished the response to the additional lighting in 12-day-old, blinded rats (*4*). This photoreceptor has not yet been identified.

The Harderian gland, first described in 1694 in deer (*5*), is located behind and around the eye (Fig. 1). It is found in all vertebrates with the possible exception of higher primates; in some instances it is larger than the eye itself. Its function is unknown; speculation has ranged from a source of lubricant for the eye (*6*) to gonadal regulation (*6*) through merocrine secretion. Reddish porphyrins (primarily protoporphyrins) are present in this gland in the rat (*7*), and we have observed fluctuations in the porphyrin of the gland under different lighting conditions. We have used the following experimental design, essentially identical to that previously used (*4*) to examine a possible connection between the Harderian gland and the pineal serotonin content.

Newborn, male and female, Long-Evans rats were kept with their mothers, under a 6 a.m. to 6 p.m. lighting schedule, in plastic cages at a constant temperature at least 4 days prior to the experiment. All rats were blinded by complete bilateral enucleation or bilateral orbital enucleation combined with complete removal of the Harderian gland, carried out under ether anesthesia when the rats were 9 days old. Animals were blinded by pressing the sides of curved eye forceps on either side of the eye to force open the eyelids and push the eye forward. The forceps were closed about the base of the eye, and the eye and the attached optic nerve were pulled forward. By pushing the forceps somewhat deeper into the optic cavity, the eye and the Harderian glands could be removed simultaneously. After removal of the

Table 1. Distribution of 12-day-old blinded rats. Twelve rats in each group.

Group	Blinded	Harderian gland removed	Maintenance in light	Time killed (p.m.)
A	+	−	+	1:00
B	+	−	−	11:00
C	+	+	+	11:00
D	+	−	+	11:00

Harderian gland, the contents of the eye cavity were also gently aspirated. Bleeding was not profuse and soon stopped. There was essentially no mortality, and in other experiments animals with the Harderian gland removed have been maintained for considerable periods of time without difficulty. The completeness of the removal was determined by examining the eye cavity for fluorescence at 366 nm after the rats were killed.

The operated suckling rats were then returned to their mothers and maintained under diurnal lighting conditions until they were 12 days old, at which time they were removed from their mothers and distributed into four groups of 12 rats 5 hours before being killed (Table 1). The rats were decapitated, and the pineals were quickly removed, weighed, homogenized in 2 ml of 0.02*N* hydrochloric acid saturated with KCL, and assayed immediately for serotonin (*8*). Three pineal glands were pooled for each sample.

Pineal serotonin is three times greater in blinded rats killed at 1 p.m. than in those killed at 11 p.m. (group A to B; $P < .05$) whereas concentrations in animals maintained in light until 11 p.m. were intermediate (group D) (Fig. 2). These results confirm the previous reports of the circadian rhythm in the pineal and the influence of light upon that rhythm. More important, however, removal of the Harderian gland (group C) abolished this influence of light. Concentrations of serotonin in the pineals of this group of animals maintained in light were identical to those in blinded animals maintained in darkness (group C versus B) and significantly lower than those in blinded animals kept in light (group C versus D; $P < .05$). Removal of the Harderian gland was thus comparable to the blinding and hooding in Snyder's experiments (*2, 3*). These results suggest that the Harderian gland may be the extraretinal receptor responsible for the maintenance of the circadian rhythm of pineal serotonin in blinded rats. However, other explanations, such as destruction of unidentified nervous or glandular tissue affecting the pineal, cannot yet be excluded.

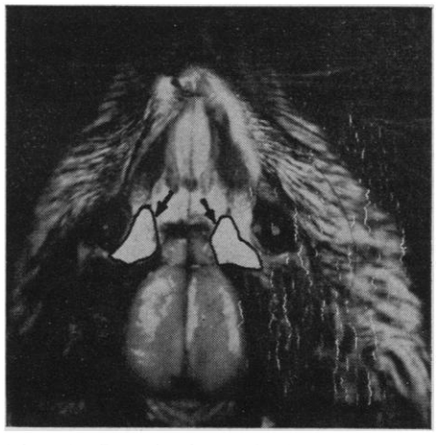

Fig. 1. Localization of the Harderian gland in relation to the eye and brain in a female rat of the Long-Evans strain.

Benoit (*9*) has observed, in the duck, that hooding prevents light stimulation of the testes and that testicular stimulation occurs if light is presented to the lateral side of the head after section of the optical nerves or if light is introduced into the eye socket after removal of orbital tissue. Since birds have well-developed Harderian glands (*10*) these results could be due to stimulation of this gland. It was also observed that visual stimulation of the testicular response in ducks was limited to red and yellow light.

The chromophore composition of the Harderian gland of the duck is unknown, but Pedler and Boyle (*11*) have shown reddish oily microdroplets of unknown origin in the retina of pigeons that, at least superficially, resemble the oily reddish material in the Harderian glands of rats. In the Harderian glands of rats, this material is composed of porphyrins having a fluorescent emission peak at 600 nm. A greenish fluorescent material of unknown compostion is also present.

The significance of a possible extraretinal photoreceptive function for the Harderian gland is still difficult to assess. One possibility is that the porphyrins in the gland permit conversion of ultraviolet frequencies below the perceptual threshold to frequencies within the visual range. Thus Granit (*12*) observed a hump at 600 nm in scotopic sensitivity curves in the rat. The visual elements of the mammalian retina face away from the incoming light but face toward the Harderian gland, which therefore may act as a reflector and transducer of light information. The role of the Harderian gland in light sensitivity and the mechanism by which it may act to affect pineal serotonin cycling—light transduction, direct neural stimulation, or endocrine function—is not known.

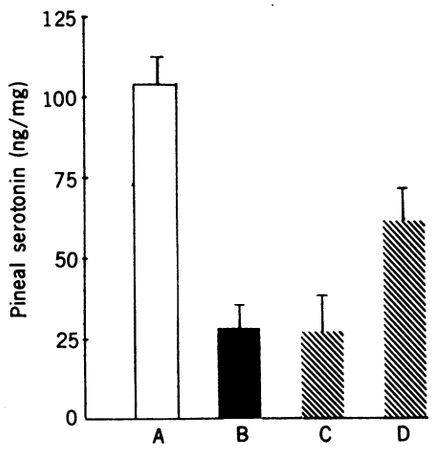

Fig. 2. Effect of removal of the Harderian gland on the serotonin content of the pineal gland in response to light in 12-day-old blinded rats. Each group contained 12 rats. Vertical bars show the standard error of the mean. (A) Killed at 1 p.m.; (B) killed at 11 p.m., maintained in dark; (C and D) killed at 11 p.m., maintained in light (Harderian gland removed from group C).

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